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Ms. Nancy T. Cherry
Scientific Advisors & Consultants Staff
Center for Biologics Evaluation and Research
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1401 Rockville Pike
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Re: January 31, 2001 Advisory Committee Meeting

Dear Ms. Cherry:

If it is at all possible, we would like to add the brief enclosed Supplemental Scientific Summary and Bibliography to our submission, which we have prepared upon consultation with, and the assistance of, expert consultants. We feel that it is important for the Committee members to consider this scientific information in their deliberations.

We would appreciate it if you could simply include this in your mailing to the Committee members. As we discussed with respect to our initial filing, we will bring plenty of copies of this, together with copies of the cited articles, along with us next week.

Thank you again for your assistance and cooperation in this matter.

Respectfully submitted,

Albert J. Brooks, Jr.

Enclosure

Supplemental Scientific Summary and Bibliography

Risks Associated with *Borrelia burgdorferi* OspA Protein Vaccination

There is strong evidence of a genetic predisposition for arthritic complications with regard to HLA-DR4 and HLA-DR2 alleles and development of OspA antibodies among Lyme disease patients. There is also considerable evidence for localized and generalized inflammatory responses to purified OspA protein, which in turn could mediate a variety of pathological changes including arthritis. Scientific publications describing these findings and their implications for vaccination with OspA protein follow.

Allen Steere's laboratories have published several articles showing that development of antibodies to the OspA protein (Infect. Immun. 61:2774 [1993], Arthritis. Rheumatol. 37:878 [1994], Infect. Immun. 63:2228 [1995], Infect. Immun. 64:1284 [1996], Science 281:703 [1998] Infect. Immun. 67:173 [1999], Arthritis Rheumatol. 42:1813 [1999]), and expression of DR4 and DR2 proteins (New Engl. J. Med. 323:219 [1990], Hum. Immunol. 31:20 [1991], Infect. Immun. 61:2774 [1993], Arthritis. Rheumatol. 37:878 [1994], Science 281:703 [1998]) are strongly correlated with development of relatively severe and treatment-resistant arthritis. These findings were considered as possible risks during FDA review of the clinical trial proposals for the Lyme disease vaccines.

Two studies involving Lyme disease have reported that 31% of humans express DR4 alleles [Steere, et al. Ann. Int. Med. 90:896 (1979); Steere, et al. New. Engl. J. Med. 323:219 (1990)] Whereas the values given for the percentage of chronic severe Lyme arthritis patients that express HLA-DR4 (or DR2) alleles in these studies were determined experimentally, the percentage of "normal" humans with these alleles was apparently derived from previous studies. Numerous subsequent studies have reported a correlation between DR4 expression and arthritis persistence and severity in Lyme disease, including the potential for autoimmune responses.

A recent paper reported several human substances that cross-react immunologically with the OspA protein [Maier, et al. Eur. J. Immunol. 30:448 (2000)]. This paper, and an earlier paper showing cross-reactivity with human LFA-1 [Gross, et al. J. Immunol. 160:1022 (1998); Gross, et al. Science 281:703 (1998)], raise legitimate concern that the OspA vaccine may indeed induce autoimmunity, as well as chronic inflammatory arthritis, particularly in people expressing HLA-DR4 alleles. A very recent paper supports these findings and provides experimental tools for assessing these effects in patients and vaccine recipients [Meyer, et al., Proc. Nat. Acad. Sci. 97:11433 (2000)]. These and other studies discussed below present a paradox for OspA vaccination, suggesting that the immune response to particular regions of the OspA protein is both the most protective against infection, and potentially the most self-destructive.

Proteins like OspA are all comprised of chains of 20 (+/-) different amino acid residues, arranged in an order encoded by genes. Each chain has two ends denoted as the N-terminus (amino) and the C-terminus (carboxy). The chains can range from a dozen or so up to hundreds of residues. OspA has approximately 273 amino acid residues, depending on the species and

strain of spirochete. The immune system recognizes and responds to small segments (epitopes) of the proteins ranging from about 4 to 10 residues. For example the segment of OspA that cross-reacts with human LFA-1 consists of residues 165-173 (numbered from the N-terminal end). We hope that the immune system can always distinguish between foreign and "self" epitopes. Autoimmunity occurs when the immune system mounts an attack against self. Prolonged autoimmunity can cause serious diseases including lupus and rheumatoid arthritis.

When the immune system first recognizes foreign material (or "antigens"), it is typical for B cells to produce M class antibodies (IgM) that recognize and bind to the antigen. This occurs in Lyme disease with OspA as well [Kalish, et al., Infect. Immun. 63:2228 (1995)]. In most infections, IgM production is followed by a class switch to IgG. However, the class switch to IgG antibodies to OspA is delayed or absent in most Lyme disease patients. It is the appearance of an IgG response to OspA that has been correlated with the onset of severe arthritis [Kalish et al, Infect. Immun. 63:2228 (1995), Akin, et al, Infect. Immun. 67:173 (1999), many others]. Such a switch is directed by T cells, which must be activated by contact of a cell-surface "T cell receptor" with a complex of other molecules including a segment of the antigen (a T cell epitope), and a class II major histocompatibility (MHC) protein like the HLA-DR4 gene products. Such T cells are referred to as "restricted" in terms of their requirement for a particular MHC gene product (they will only respond to a particular antigen if is bound to the correct MHC protein). Activated T cells largely orchestrate the immune system's response to the foreign antigen.

It is now believed that chronic (treatment resistant) Lyme arthritis is associated with DR4-restricted T cells, which recognize and become activated by one or more central (residues 84-113) and C-terminal (residues 133-273) fragment(s) of OspA presented in association with HLA-DR4 MHC proteins [Kamradt, et al, Infect. Immun. 64:1284 (1996)]. Paradoxically these are the same regions of OspA that elicit protective immunity [Schaible, et al. Proc. Nat. Acad. Sci. (USA) 87:3768 (1990), Sears, et al. J. Immunol. 147:1995 (1991), Bockenstedt, et al., J. Immunol. 151:900 (1993), Kurtenbach, et al. Vaccine 15:1670 (1997). This stimulation causes the T cells to initiate a type Th1, inflammatory cytokine (Interleukin 1 (IL-1), IL-6, IL-12) response [Infante-Duarte and Kamradt, Infect Immun. 65:4094 (1997); Yin, et al. Arthrit. Rheum. 40:69 (1997)]. Resulting inflammation attracts other leukocytes (white blood cells including neutrophils, monocytes, macrophages, etc.) causing release of a variety of factors such as nitric oxide (NO), interferon-gamma (IFN), and tumor necrosis factor-alpha (TNF) capable of damaging surrounding tissue. Such cells can carry LFA-1 and possibly other molecules with cross-reactive epitopes, essentially amplifying and sustaining a self-damaging immune response, even if the OspA protein that initiated the response is eventually removed from the system [Gross et al. Science 281:703 (1998), Maier, et al, Eur. J. Immunol. 30:448 (2000)]. Maier and co-workers demonstrated that DR4-restricted T cells exhibiting T cell receptors that recognize just two of several potential epitopes on OspA (residues 164-175 and 235-246) can cross-react with at least 9 and 7 different human proteins, respectively. They qualify these results by noting that the potential for cross-reactivity, does not necessarily guarantee deleterious effects, as many other factors may influence development of autoimmunity.

Within three years after the 1982 publication establishing *B. burgdorferi* as the infectious agent of Lyme disease, Gail Habicht and co-workers [J. Immunol. 134:3147 (1985), Ann. NY Acad. Sci. 539:80 (1988), J. Rheumatol. 16:800 (1989)] showed that IL-1, was produced by human blood monocytes in response to *B. burgdorferi*, and suggested that IL-1 could be responsible for inflammation and arthritis in Lyme disease. They also showed induction and secretion of IL-6, another inflammatory cytokine, by human glial cells [J. Infect. Dis. 164:568 (1991)]. Later a string of papers from Janice Weis' laboratory demonstrated that OspA protein is a potent inducer of IL-1, IL-6, and a variety of other inflammation mediators including TNF, IFN, NO, and IL-12 [Infect. Immun. 61:3843 (1993), Infect. Immun. 62:520 (1994), Infect. Immun. 62:3663 (1994), J. Immunol. 157:4584 (1996)]. Concurrently Weis' lab [Infect. Immun. 60:3033 (1992), Infect. Immun. 62:463 (1994)] and Whitmire and Garon [Infect. Immun. 61:3843 (1993)] showed that *B. burgdorferi* antigens, and the OspA protein in particular, induce blood B lymphocytes (B cells) to proliferate and non-specifically secrete IgM and IgG class antibodies. These factors vary in their localized and/or systemic effects. Furthermore varying levels of some of these factors can modulate the production and effects of other factors. In general elevated levels of IL-1, IL-6, IFN, and NO have been associated with arthritogenesis, and IL-6 and IFN have been associated with neurological damage in Lyme disease. Unregulated antibody production can be relatively benign, or can lead to immune complex disease often manifested by arthritis and/or nephritis (kidney damage) depending on the binding specificities of the antibody molecules produced.

One important factor to consider in interpreting these experimental findings is the concentration of OspA protein needed to induce such responses. Weis and co-workers reported B cell activation (proliferation and unregulated antibody-secretion) and inflammatory responses using concentrations of 10 nanograms of OspA protein per milliliter of buffer. This is approximately 6000 fold less concentrated than the vaccine preparation, which contains 30 micrograms of OspA protein in a volume of 0.5 milliliters. Thus it is not surprising that localized pain and inflammation was reported following injections. With time, the inoculation would be expected to dissipate to concentrations below the experimental threshold of pathogenic effects. However, it is possible, if not likely, that dissemination of inflammatory mediators induced by an initial localized response could cause systemic effects.

Studies have shown that the inflammatory cytokine IL-6 is secreted by human endothelial cells (cells that line the interior walls of blood vessels) in response to stimulation by OspA protein [J Immunol. 157:4584 (1996), J. Immunol. 160:5485 (1998)]. Similarly OspA induces human blood monocytes to secrete IL-6 [Infect. Immun. 67:140 (1999), and possibly IL-1, IFN, and NO as well [mouse studies: Infect. Immun. 62:3663 (1994), Infect. Immun. 62:4632 (1994), Infect. Immun. 63:3886 (1995), Infect. Immun. 67:140 (1999), Infect. Immun. 67:5142 (1999)]. These molecules circulate and mediate inflammatory damage systemically. It is known that levels of IL-1 and IL-6 can be elevated in the serum and cerebrospinal fluid of Lyme disease patients. Weller and co-workers showed that levels of IL-6 can parallel disease activity [Arch. Neurol. 48:837 (1991)]. This finding was further supported in mouse models [Microbiol.

Immunol. 41:427 (1997), Infect. Immun. 67:5142 (1999)]. In the latter paper, Brown and co-workers concluded that the relative levels of IL-6, TNF, and NO versus the anti-inflammatory cytokine IL-10 are useful in predicting the extent of inflammatory arthritis in mice.

Although reports of controlled studies examining the predictive value of inflammatory mediator levels in human Lyme disease are lacking, measurement of IL-6, and possibly IL-1, TNF, NO, and IL-10 levels in vaccinated individuals could be useful for assessing inflammatory response to the OspA inoculum. Such assessments of inflammatory mediators would add significant expense in a large pool of vaccine recipients, particularly considering that samples would need to be measured at time points before injection, within 24 to 48 hours after injection, and at one or more subsequent post-injection, and post-booster periods.

Conversely, unregulated antibody over-production (hypergammaglobulinaemia) can be measured fairly inexpensively. Vaccination causes an increase in measurable antibody response specifically against the intended antigen, in this case OspA protein. This increase predictably begins within several days to weeks after injection, but only marginally increases total serum antibody concentration, particularly after the first and second injections. In contrast, non-specific antibody production induced by OspA causes a rapid 2-3 day increase in total antibody secretion and B cell proliferation. Furthermore the antibody production is largely non-specific for the OspA protein, meaning that the antibodies produced could essentially, and randomly, recognize and attack any of possibly millions of different antigens. Such antigens could include "self" or auto-antigens. The recent report of cross-reactive antibodies that bind to both OspA and LFA-1 would be an example of distinct, but equally dangerous OspA-specific yet cross-reactive auto-antibodies. Virtually all commercial medical laboratories could distinguish between OspA-specific and non-specific serum antibody concentrations. Such B cell stimulation and non-specific antibody secretion could induce additional auto-antibodies, which if effective at a binding to self antigens would be expected to cause direct damage to cells and tissues, and long term immune complex diseases such as arthritis and nephritis.

Since nearly one-third of potential recipients can be expected to carry HLA-DR4 alleles, strong published evidence supports testing all potential vaccine recipients for these gene products, and for advising DR4-positive individuals of possible increased risk for post-vaccination complications. The cost of such testing has been reported as approximately $300 per patient. However, the findings of studies showing the potential for inducing what Dr. Steere terms severe treatment-resistant arthritis warrant careful screening and monitoring of individuals seeking the OspA vaccine.

Submitted by Sheller, Ludwig & Badey
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